Some Biological Effects of the Flavonoids*

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There are known at present at least 137 natural flavonoids, occurring in at least 62 families, 153 genera, and 277 species of plants. About 33 different types of physiological and biochemical activities have been reported for one or another of 30 of these flavonoids. This paper summarizes these activities and what is known about the relation of structure to such activities. It is suggested that this is a fruitful field for further research.

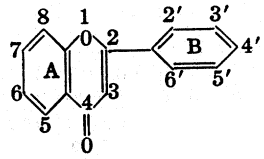
THE PURPOSE of this article is to collect the scattered evidence for the biological activities of the various flavonoids in the hope that the diversity and importance of some of their activities will stimulate further scrutiny of this class of compounds. The literature reveals that at least 33 different manifestations of activity of one or another of the flavonoids have been reported. These activities are listed in Table I. No attempt is made here to evaluate or to review critically these published findings.

REVIEW

Probably the first record of physiological activity was that of Koike in 1931 (26) on the diuretic effect of seven flavonoids. During the next fifteen years a few reports of other effects appeared. From 1947 on there has been considerable activity in this field. Rutin was developed as a commercial drug, then its quercetin moiety was also shown to be active in correcting capillary fault (21). Quercitrin and, to a lesser extent, quercetin were shown to have an antiviral effect (13). Most dramatic of all, the dread subterranean clover disease of Australia, causing sterility in sheep, was proved to be due to the estrogenic effect of genistein and formonetin (formononetin) (5).

To keep the scope of this review within reasonable limits, it will be confined largely to the aglycones themselves and will not deal with their glycosides.

For convenience, the skeleton of the flavonoids is given below, since it will be necessary frequently to refer to position numbers. The structure given is for the flavones. In the flavanones the 2,3 double bond is missing, with a hydrogen at 2 and two hydrogens or their equivalent on 3. In the isoflavones ring B is attached at 3. In the chalcones the pyrone is open, the oxygen at 1 becoming an OH.



About 30 flavonoid aglycones have been reported as having various activities on organisms, tissues, and enzymes or effects on physiological functions. Table II lists these, together with the kinds of activities established for each.

It will be noted in Table I that the types of activity are extremely diverse. Furthermore, different flavonoids may have opposite effects: depress or stimulate the heart; decrease or increase blood

* Received December 20, 1954, from the Eastern Regional Research Laboratory, Agricultural Research Service, United States Department of Agriculture, Philadelphia 18, Pa.

TABLE I.—TYPES OF BIOLOGICAL AND CHEMICAL ACTIVITY REPORTED FOR FLAVONOIDS

1. Estrogenic
2. Bactericidal
3. Spermicidal
4. Tumor reduction
5. Oxido-reduction of cytochrome c
6. Toxic to grapefruit albedo
7. Toxic to fish
8. Anthelmintic
9. Heart stimulant
10. Heart depressant
11. Diuretic
12. Strengthening of capillaries
13. Protective against irradiation
14. Contraction of uterus
15. Inhibition of muscle action
16. Potentiation of epinephrine
17. Hypotensive
18. Hypertensive
19. Inhibition of antibiotic
20. Inhibition of bactericides
21. Inhibition of oxidation in fat
22. Activation of enzymes
23. Inhibition of enzymes
24. Gynotermone
25. Reduction of iodine in thyroid
26. Antihistamine
27. Sparing of ascorbic acid
28. Increase of respiratory movement
29. Antivirus
30. Cathartic
31. Protection against frostbite
32. Increase in alkaline reserve
33. Protection of islets of Langerhans

TABLE II.—NATURALLY OCCURRING FLAVONOIDS HAVING BIOLOGICAL EFFECTS

	Position of the OH Groups	Position of the CH_3O Groups	Types of Biological Activity[a]
	Flavonols		
Calycopterin[b]	4′,5	3,6,7,8	7, 8, 14, 15, 17, 28
Chrysin	5,7	..	7, 14, 15, 17, 28
Flavone	..	..	2
Galangin	3,5,7	..	7
Genkwanin[c]	4′,5	7	14, 15, 17, 28
Gossypetin	3,3′,4′,5,7,8	..	12, 16
Isorhamnetin	3,4′,5,7	3′	24
Kaempferol	3,4′,5,7	..	7, 11, 30
Luteolin	3′,4′,5,7	..	22
Morin	2′,3,4′,5,7	..	2, 11, 13, 16, 23
Myricetin[d]	3,3′,4′,5,5′,7	..	7, 9, 11, 18
Primuletin[e]	5	..	16
Quercetagetin	3,3′,4′,5,6,7	..	16
Quercetin	3,3′,4′,5,7	..	3, 4, 5, 7, 9, 12, 16, 19–23, 25, 26, 29, 31
Rhamnetin	3,3′,4′,5	7	2, 9, 17, 23
Tricin	4′,5,7	3′,5′	15
Ulexflavone	Unknown		11
	Isoflavonols		
Biochanin A	5,7	4′	1
Daidzein	4′,7	..	1
Formonetin[f]	7	4′	1
Genistein[g]	4′,5,7	..	1
Santal	3′,4′,5	7	15
Tectorigenin	4′,5,7	6	11
	Flavanols		
Butin	3′,4′,7	..	7
Catechin[h]	3,3′,4′,5,7	..	3, 13, 21, 23, 27, 31, 33
Epicatechin	3,3′,4′,5,7	..	32
Eriodictyol	3′,4′,5,7	..	11, 23
Hesperetin	3′,5,7	4′	10, 13, 21
Homoeriodictyol	4′,5,7	3′	11, 13, 23
Naringenin	4′,5,7	..	6, 17, 21

[a] Numbers under biological activity refer to Table I.
Synonymous with: [b] Thapsin. [c] Puddmetin. [d] Cannabiscetin. [e] 5-Hydroxy flavone. [f] Formononetin, ononein.
[g] Prunetol. [h] Dihydroquercetin, distyllin, taxifolin.

pressure; depress or stimulate enzymes. Since the one structural item which the flavonoids have in common is the phenylchromone nucleus, the varied biological properties must be due to the substituents on the 10 or 12 sites where derivatives usually occur.

A few attempts have been made to fix the structure imparting a given activity. Von Jeney and Czimmer (24) state that rhamnetin and quercetin had a favorable effect on fatigued or poisoned frog heart, while hesperetin had an adverse effect. They believe this is because the 4′ site carried OH in the first two and hence is available for biological oxidation, while in hesperetin the 4′ is occupied by CH_3O. The authors ignore, however, the fact that hesperetin is also a flavanol and the others are flavonols.

The different action of flavanone and flavone derivatives was brought out in comparative studies of toxicity to fish (43). The CH_3O derivatives of flavanones were less toxic than the corresponding flavone compounds, but with OH derivatives the reverse was true. In an earlier paper (41) it was postulated that a pyrone ring containing the structure CO—C=C—O— is a toxophore. This does not help much, since positions 1 through 4 comprise this structure in all the flavones. Butin became far more toxic when it was converted to its chalcone, butein (45).

Probably the most clean-cut deductions concerning structure and activity were those of Clark and Geissman (9) on potentiation of epinephrine effects.

On the basis of the results of tests of 70 natural and synthetic flavonoids, they predicted a structure of high activity, synthesized it, and confirmed the prediction. It was 3,3′,4′-trihydroxyflavone, which has not as yet been found in nature.

Wilson and DeEds (63) arrived at similar deductions on the effect of structure in protecting epinephrine against destruction. "On an equimolecular basis, it appears that a glycosidal linkage at the 3-position does not modify activity, that a glycosidal linkage at position 7 increases activity, while methoxylation at 7 decreases activity, that a 2-3 double bond increases activity, and that 2 free OH groups ortho to each other on the benzene ring increase activity."

Szent-Györgyi (58) also found that the *o*-di OH structure could catalyze oxidation of ascorbic acid by peroxidase, probably through quinone formation. On the other hand, Suzuki and Mori (56) reported that some flavonoids inhibited oxidation of ascorbic acid, and ascribed the action again to the *o*-di OH groups. Since the enzyme-substrate systems in the two cases were different, it is possible that there is no real discrepancy. In any event, quinone formation by virtue of adjacent OH groups seems to be required for these oxidation-reduction activities.

The importance of the 4′ position showed up again in a comparison of the action of flavonoids on bovine and streptococcal hyaluronidase (47). Free OH groups at both 3′ and 4′ were necessary. Action on three other enzymes followed about the same pattern.

Beiler, *et al.* (3), found that choline acetylase was inhibited by rutin, catechin, epicatechin, quercetin, esculetin, and morin, but not by hesperidin methylchalcone, neohesperidin, naringin, or esculin. Their explanation is: "The structure of quercetin is given below, the active quinone-forming groups being the 3′,4′ di OH and the 3-OH 4-keto. In rutin, the 3-position is blocked, and the activity is about 0.1 that of quercetin. Catechin has no keto group at 4, and the activity is again of the order of 0.1 that of quercetin. In morin, on the other hand, the dihydroxy grouping is 2′,4′, thus precluding quinone formation, and the activity is about the same as that of quercetin. The 3,4 grouping is thus apparently much more active than the 3′,4′."

Similarly they (38) explained the comparative action of flavonoids on histidine decarboxylase.

The necessity of substituents which can readily form quinones was also indicated for spermicidal action (57).

Kuhn, *et al.* (28), discovered the remarkable gynotermone effect of isorhamnetin. At the amazingly low concentration of 1 to 400 billion (about 2.8 molecules per cell) it would convert the bisexual cells of the alga *Chlamydomonas* to the female form and enable it to conjugate with a male gamete only. This action is so specific that it was possessed in not the slightest degree by 35 other flavonoids, many of them derivatives of isorhamnetin and differing only in a CH_3O or an OH on any one of five sites of the nucleus. Furthermore, the 3,4-diglucoside of isorhamnetin immobilized the gametes (27).

Both hypo- and hypertensive actions have been reported, but so far no definite relation between these actions and structure can be discerned. In one series (2) two flavonols and one flavanol were hypertensive, and three flavonols were inactive. In another (37), the three flavonols tested were hypertensive. In a third (16), the two flavonols tested were hypotensive.

Reports published on other actions of flavonoids are even more sketchy, being limited to one or two papers. They illustrate, however, the extremely diverse properties of these compounds. Some are bacteriostatic (25, 59) or bactericidal (59, 25, 42) depending on concentration. Quercetin inhibits the antibacterial activity of dicoumarol (42). The action of tomatine on *Candida albicans* (35) is a spermicide (57) and has some antiviral action (13). Calycopterin is anthelmintic (46).

EFFECTS

A variety of effects on certain animal and plant tissues have been reported: reduction of a tumor (34); stimulation of the heart (24, 16) and the opposite (24); strengthening of capillaries (21), the so-called vitamin P effect; contraction of uterus (37); inhibition of muscle action (37, 14); reduction of iodine in the thyroid (12); increase in repiratory movement (37); necrosis in grapefruit albedo (22); protection against frostbite (1); increase in alkaline reserve (32); protection of islets of Langerhans on diets low in ascorbic acid (18).

Some other effects have been noted: reduction of cytochrome c (11); diuretic action (16, 17); protection against x-rays (15) and radium (53); and possibly against ultraviolet light in plants (52); potentiation of epinephrine (9, 63, 30); antioxidant in fat (29); activation of enzymes (31); inhibition of enzymes (47, 3, 38, 4); protection against histamine shock (62); sparing of ascorbic acid (10, 23, 55).

The question naturally occurs as to whether, in these biological manifestations, it is the aglycone or the glycoside as a whole which is active. Some comparisons are reported, and apparently the answer is that sometimes it is the one, sometimes the other, sometimes either one. Thus, against virus (13), rutin was more active than its aglycone, quercetin. In inhibiting the bacteriostatic action of dicoumarol (42) the decreasing order of activity was rutin, quercitrin, and quercetin. In protection against x-rays (15) rutin seemed to be effective and quercetin ineffective. On the other hand, in inhibition of histidine decarboxylase only aglycones were effective (38). In correcting capillary fault (21) both rutin and quercetin are active, but the latter is more generally effective. In inhibition of hyaluronidase (47) and in spermicidal effects (57) the aglycones were more active than the glycosides. In causing deterioration in grapefruit albedo naringenin was 1,000 times as active as naringin (22).

DISCUSSION

As mentioned above, widespread sterility in sheep has been caused by their eating too much *Trifolium subterranium*. Bradbury and White were able to fix the blame on genistein and, to a lesser extent, on formonetin (5). After a further study on mice (6) they came to the following generalizations: "The estrogenic activity of the isoflavones is not analogous to that of the stilbestrol series. A 5-OH group (chelated with the carbonyl group) appears to be essen-

tial for activity while 2-alkyl substituents greatly reduce the activity, probably owing to distortion of the planar ring system of genistein, which is indicated by models and the effect of these substances on the ultraviolet absorption spectra of the isoflavones.... In contrast, the isoflav-3-ens are estrogenic only when they have a 2- or 4-substituent and are then much more active than genistein. Furthermore the active compounds have no 5-OH group and appear to be strictly analogous to compounds of the stilbestrol series in their estrogenic activity." Solmssen (54) points out that in synthetic estrogens there is a relation between activity and number and arrangement of OH groups. Cheng, *et al.*, confirmed the estrogenic action of genistein and formonetin (7) and added biochanin A and daidzein to the list (8). Legg, *et al.* (33), demonstrated estrogenic action in *Lolium perenne*, *Dactylis glomerata*, and *Trifolium pratense*, but no attempt was made to connect the activity with flavonoids, although four of the latter are known to occur in *T. pratense*. It would seem that the estrogenic action of these compounds should be studied further.

What happens to the flavonoids when taken into the body of higher animals? In general it has been reported that ingested flavonoids are excreted in the urine unchanged (e. g., 19, 50). Recently, however, Murray, *et al.* (40), report that, when a rabbit is given quercetin, within a few hours an appreciable proportion of it appears in the urine as 3,4-dihydroxyphenylacetic acid. This raises at least one intriguing question: is the therapeutic value of quercetin due to itself or to its metabolic products?

CONCLUSIONS

Thus, when this scattered information is brought together we see a diversity of physiological and biochemical activity manifested by a single group of naturally occurring compounds. The possibilities of further exploration in this field can be shown by the statistics involved. To date about 33 kinds of activity have been demonstrated for one or more of about 30 flavonoids. There are now known about 137 different natural flavonoids—22 isoflavonoids, 73 flavonols, 33 flavanols, and 9 of unknown structure. These occur in at least 62 families, 153 genera, and 277 species of plants. A great variety of derivatives can be prepared. There are a number of useful reviews on the chemistry and occurrence of the flavonoids (20, 36, 39, 48, 51, 61).

As to concentration in the plant sources, in most cases the flavonoids appear to be but a few per cent of the dry weight. On the other hand, 15 different flavonoids have been found in wood or bark of 25 species of lumber trees of the pine family and others in hardwoods. Thus the total quantity available here would be large, even though the concentration might be low.

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